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Antioxidant properties of essential oils extracted from three species of Moroccan junipers

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ABSTRACT

Essential oils from *Juniperus thurifera, Juniperus oxycedrus* and *Juniperus phoenicea* (Cupressaceae) collected in various areas in Morocco were extracted by hydrodistillation and analyzed by CG and CG/SM. Twenty-four components were identified in the essential oils from the branches of *Juniperus thurifera*, forty seven from *Juniperus oxycedrus* and twenty-six from *Juniperus phoenicea*. The majority components obtained are pinenes and especially β-pinene (36.3%) for the essential oils from the branches of *Juniperus thurifera* and α-pinene for those of *Juniperus oxycedrus* (52.1%) and *Juniperus phoenicea* (64.2%). The antioxidant properties of oils were determined by the DPPH method and were compared to that found for the reference compound (BHT) and for other essential oils.

KEYWORDS

Juniperus thurifera;
Juniperus oxycedrus;
Juniperus phoenicea;
Essential oils;
Antioxidant activity.

INTRODUCTION

The phenomenon of self-oxidation of the lipids produced during the processes of conservation and transformation of food is a very complex purely chemical process, involved in radicalization reactions able to self-maintaining and requiring only the presence of atmospheric oxygen. It is responsible of the formation of chemical compounds harmful for the health of the animals as well as men[1].

An antioxidant is a substance which, added to low dose with a naturally oxydable product with the air, is able to slow down the phenomenon of oxidation by increasing the time leading to a detectable deterioration of the product[2]. As synthetic antioxidants generally used in agribusiness industries are currently questioned in reason of potential toxicological risks (e.g., buthylhydroxyanisol (BHA), buthylhydroxytoluene (BHT) and tertiobutylhydroquinone (TBHQ))[3,4], new vegetable sources of natural antioxidant s are thus searched.

Many works, in particular those of Chimi[5,6], attest the role of some phenolic compounds extracted from virgin olive oils on the good resistance to radicalizing oxidation : the major compounds are oleuropeine, tyrosol, hydroxytyrosol and cafeic

acid[7]. In this context, the antioxidant properties of the volatile extracts from aromatic plants were largely studied in the aim of protection against the unsaturated fatty acids from animal origin[8]. Thus, an extraction using supercritical CO_2 allowed Djamarti and al. (1991)[9] to obtain from rosemary and sage an extract rich in carnosol and ramanol, molecules whose antioxidant capacity can be equivalent or even higher than that of BHT.

In this context, the aim of the present work is to evaluate the antioxidant activity of essential oils from the branches of *Juniperus thurifera*, *J. oxycedrus* and *J. phoenicea* collected in various areas of Morocco for the valorization of these three aromatic plants. A comparison with the value found for the compound of reference buthylhydroxytoluene (BHT) was made.

MATERIALS AND METHODS

Plant material

The aerial parts (stems and leaves) of *Juniperus thurifera* (thuriferous juniper) and *J. oxycedrus* were collected in March (2008). The samples of thuriferous juniper come from Jbel Bouiblane in Eastern Middle-Atlas (Morocco) and those of the *J. oxycedrus* from Taffert forest in Eastern Middle-Atlas (Morocco). The harvest of the samples of *Juniperus phoenicea* (red juniper) was carried out during Mars (2009) in Aghbar forest (High Atlas, Morocco). The taxonomic identification of plant materials was confirmed by A. Aafi, botanist from the Center of forest Research of Rabat, Morocco.

Distillation of essential oils

The isolation of essential oils was achieved by hydrodistillation that was carried out using a Clevenger-type distillation system[10]. The distillation of each sample lasted approximately 4 hours. For each distillation, 200g of fresh raw material was used. Previously, the moisture of the samples was measured in order to calculate the essential oil yields corresponding to 100g of dry matter. Essential oils were dried over anhydrous sodium sulphate and stored at 4°C in the dark until testing and analyzing.

Chromatographic analyses

Gas Chromatographic (GC) analyses were performed with a Hewlett-Packard (HP 6890), equipped with a capillary column HP-5 (30m × 0.25mm, 0.25 µm film thickness) and a detector FID at 250°C provided by H2/Air gas mixture and split-splitless injector heated at 250°C. The injection mode is split (with a split ratio of 1/50 and flow at 66 ml/min). The vector gas used is N2 with 1.7 ml/min. The column temperature was programmed from 50°-200°C at 4°C/min. The apparatus was guided by a computer system type "HP ChemStation," which manages the apparatus functioning and allows monitoring of chromatography analyses. The injected volume was about 1 µl. The identification of constituents was achieved on the basis of retention indices and gas chromatography/mass spectrometry (GC/MS). The GC/MS analyses were performed on a Hewlett-Packard (HP 6890) coupled with a mass spectrometer (HP 5973). Fragmentation was by electron impact under 70 eV field. The column used was a capillary column HP-5MS (5% phenyl methyl siloxane: 30 m × 0.25 mm, 0.25 µm film thickness). The vector gas was Helium with 1.5 ml/min. The column temperature was programmed from 50°-200°C at 4°C/min. The oil components were identified by comparing the retention indices of authentic materials with those of substances present in the mixture and by further confirming their identities MS (library of NIST 98 spectra).

Antioxidant activity

To evaluate the antioxidant activity, the method of 1,1-diphenyl-2-picrylhydrazyl (DPPH) as described by Leitao (2002) and Chen (2004) was used[11,12]. The experiment was carried out in a spectrophotometer (UV/visible) monochromatic (Littrow 1200 lines/mm per stage) of type M550 Camspec, controlled by a computing system with a bidirectional numerical interface (RS232C) and a screen of posting LCD backlit (1/4 VGA 320 X 240 pixels) at 517 nm. We prepared the solution of DPPH by the dissolution of 4 mg of the powder in 100 ml of ethanol (EtOH). The samples of essential oils were prepared in EtOH at a rate of 20 µg/ ml. These solutions, then underwent dilutions to obtain the follow-

ing concentrations: 1.25, 2.5, 5, 10, 20 and 40 µg/ml and the test was carried out by mixing 4 ml of DPPH solution with 1 ml of essential oils to be tested with various concentrations. The reference antioxidant or positive control (BHT) was also prepared according to the same method. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition free radical DPPH in percent (I %) was calculated as follows[12, 13] :

$$I\% = 100 \times (A_{blank} - A_{test})/A_{blank}$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{test} is the absorbance of the test compound. The graph of the percentages of inhibition according to the concentration of essential oil makes it possible to determine the IC50 and the value obtained is compared with that found for the compound of reference (BHT).

RESULTS AND DISCUSSION

Yields and chemical composition

The branches of *Juniperus thurifera* provided the highest yield of essential oils with approximately 1.32% compared to only 0.48% for those from *J. phoenicea* and 0.15% for those from *J. oxycedrus*. The rate provided by *J. thurifera* is still higher than that obtained by Akkad and al. (2001)[14] for samples of fruits, leaves and wood from this species and which did not exceed 0.8%. For *J. oxycedrus*, this rate is lower than that found for the same species in Lebanon (0.72%)[15] and Spain (1.14%)[16]. In contrast, the rate provided by *J. phoenicea*, remains higher than those in Greece (0.21%), Spain (0.30%), Egypt (0.36%) and Portugal (0.41%)[17,18].

The compounds identified in essential oils from the branches of three species of junipers are listed in TABLE 1. Twenty for constituents were detected from branches of *J. thurifera*, 47 of *J. oxycedrus* and 26 of *J. phoenicea*. In the thuriferous juniper, the majority compounds of essential oils include the β-pinene (36.26), in addition to the terpin-4-ol (12.76%), pinene α-oxide (10.89%), piperitone (10%), β-E-ocymene (4.38%), α-pinene (3.14%) and myrcene (3.13%) (Figure 1). Those from *J. oxycedrus* and *J. phoenicea* are dominated by α-pinene with respective rates of 52.13 and 64.19%. We also note the presence in outstanding quantity of limonene (7.32%), α-phellandrene (5.59%), 14-hydroxy-9-epi-E-caryophyllene (5.44%) and germacrene D (4%) in the essential oils from branches of *J. oxycedrus* and δ-3-careens (14.84%), myrcene (2.69%) and fenchone (2.12%) in essential oils of *J. phoenicea* (Figures 2 and 3). In addition, each species shows certain specificities in terpenic compounds such as the terpin-4-ol (12.76%), pinene α-oxide (10.89%) and β-E-ocymene (4.38%) for the essential oils from *J. thurifera*, 14-hydroxy-9-epi-E-caryophyllene (5.44%), β-cymene (1.56%) and manoyl oxide (1.82%) for essential oils from *J. oxycedrus* and fenchone (2.12%), camphor (0.30%) and citronellol (0.71%) for essential oils from *J. phoenicea*. By their chemical composition, our essential oils extracted from *J. thurifera* branches are richer in monoterpenes (97.09%) compared to that obtained from fruits of this species studied in Morocco by Akkad and al. (2001) (approximately 60%)[14]. They are also qualitatively similar to that of *J. communis* of Lithuania and whose principal components identified are β-pinene (39.7%), terpin-4-ol (11.8%), in addition to α-pinene (2.1%) and myrcene (2.0%)[19].

The chemical composition of the essential oil from branches of *J. oxycedrus* is close to those obtained from certain localities in Spain, studied by Velasco-Eneguerula and al. (2003) [20] of which principal components was α-pinene (59.8% - 86.9%), limonene (1.2% - 2.8%) and D germacrene (0.4% - 3.6%). Our results are slightly different from those of Salido and al. (2002)[16], Adams and al. (1999)[21] and Adams (2000)[22] who found for this species smaller quantities of α-pinene, respectively (40%), (5.1%) and (20.7%), while the rates of D germacrene and manoyl-oxide were much more important.

Another study of *J. oxycedrus* in Lebanon showed that essential oils also contains as majority components α- pinene with a rate of (27.4%), in addition to myrcene (18.9%), α-phellandrene (7.1%) and limonene (6.7%)[15].

TABLE 1 : Centesimal chemical composition of essential oils in the branches of *juniperus thurifera, J. oxycedrus* and J. *phoenicea* of Morocco

IK	Compound	*J. thurifera (%)*	*J. oxycedrus (%)*	*J. phoenicea (%)*
926	Tricyclene	-	0.2	0.2
929	α-thujene	1.5	-	-
939	α-pinene	3.2	52.1	64.2
951	α-fenchene	-	0.6	-
953	Camphene	-	0.2	1.2
957	Thuja-2-4-(10) diene	-	0.2	-
967	Verbinene	-	0.3	0.1
976	Sabinene	-	0.4	-
981	β-pinene	36.3	2.7	0.9
992	Myrcene	3.1	0.3	2.7
1001	δ -2-carene	-	1.2	-
1005	α-phellandrene	0.8	5.6	-
1010	δ -3-carene	2.3	-	14.8
1021	Orto-cymene	1.6	-	-
1025	β-cymene	-	1.6	-
1031	Limonene	-	7.3	0.4
1031	β-phellandrene	1.2	-	1.6
1039	β-Z-ocymene	1.2	-	-
1048	β-E-ocymene	4.4	-	-
1059	γ-terpinene	-	0.1	0.5
1060	α-terpinene	1.3	-	-
1066	Fenchone	-	-	2.1
1088	Terpinolene	1.9	0.8	-
1094	pinene α-oxide	10.9	-	-
1120	Trans-pinon-2-ol	0.8	-	-
1125	α-campholenol	-	0.7	-
1140	Trans-pinocarvelol	-	0.4	-
1141	Pinene trans-hydrate	0.6	-	-
1143	Camphre	-	-	0.3
1144	Trans-verbenol	-	0.3	-
1166	δ -terpineol	-	0.3	-
1172	Terpin-4-ol	12.8	-	-
1178	Terpinen-4-ol	-	0.3	-
1189	α -terpineol	1.7	0.4	0.5
1196	Verbanol	-	0.6	-
1228	Citronellol	-	-	0.7
1250	Piperitone	10	-	0.5
1272	thujyl Neo-3-acetate	0.7	0.2	-
1282	verbenyl Cis-acetate	-	0.2	-
1314	terpenyl Trans dehydro-α-acetate	0.7	-	-
1349	terpenyl A-acetate	0.5	-	-
1351	α -cubebene	-	0.1	-

IK	Compound	*J. thurifera (%)*	*J. oxycedrus (%)*	*J. phoenicea (%)*
1377	α -copaene	0.7	0.5	-
1420	E-β-caryophelene	-	0.5	1.4
1453	α-humulene	-	0.4	0.5
1477	γ-munrolene	-	0.3	0.4
1480	Germacrene D	-	4.0	1.2
1494	2-tridecanone	-	1.1	-
1499	α-munrolene	-	0.3	-
1513	γ-cadinene	-	0.7	0.2
1524	β-cadinene	-	0.5	1.4
1539	α-cadinene	1.3	-	-
1549	Elemol	-	-	0.6
1557	Germacrene B	0.4	0.3	1.5
1583	caryophyllene Oxide	-	0.3	0.3
1612	Epi-cedrol	-	1.5	-
1626	1-epi-cubenol	-	0.2	0.9
1653	α-cadinol	-	0.3	t
1663	14-hydroxy-9-epi-E-caryophyllene	-	5.4	-
1675	5-neo-cedranol	-	0.2	-
1681	Cis-14-munrol-5-eu-4-one	-	0.8	-
1690	juniper Camphre	-	1.3	0.3
1707	14-hydroxy-α -humulene	-	0.3	-
1713	(Z,Z)-farnesol	-	0.8	-
1735	Khusimol	-	0.4	-
1990	manoyl Oxide	-	1.8	-

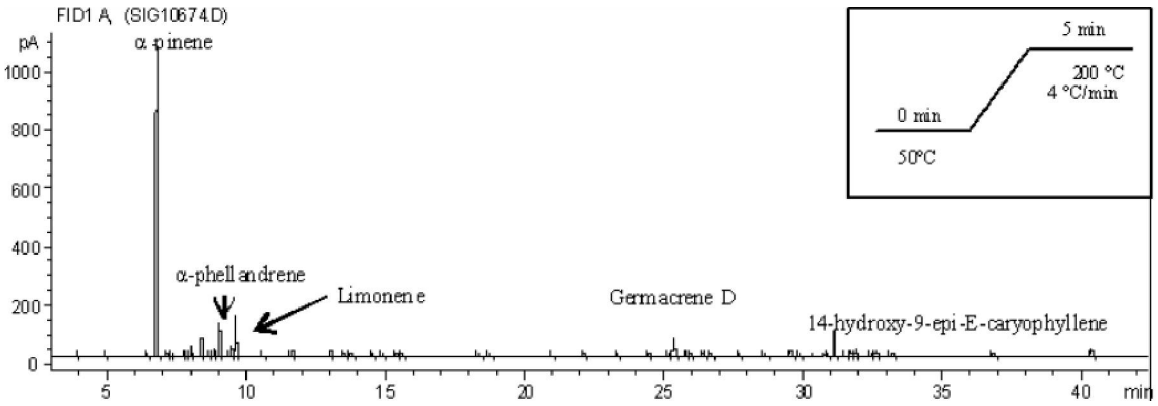

Figure 1 : Chromatographic profiles of essential oils in the branches of *juniperus thurifera*

The essential oils extracted from branches of *J. phoenicea* are richer in α-pinene (64,19%) compared to that obtained from leaves of the same species studied by Achak and al. (2008) in Marrakech regions (Morocco), and whose content for this compound did not exceed 36%[23]. They also differ by their chemical composition of essential oils from red juniper in Tunisia, Greece and Spain whose content α-pinene is also less important (38.20, 41.8 and 53.5% respectively). On the other hand, we find in the latter more myrcene (4.7, 4.5 and 4% respectively), β-phellandrene (2.1, 3.5 and 5.9 % respectively)[17, 24]. Oils in subspecies *turbinata* in Spain and *Eumediterranea* in Portugal[17] have α-pinene contents even lower (28.3 and 34.1% respectively), but much higher percentages for β-phellandrene (25.3

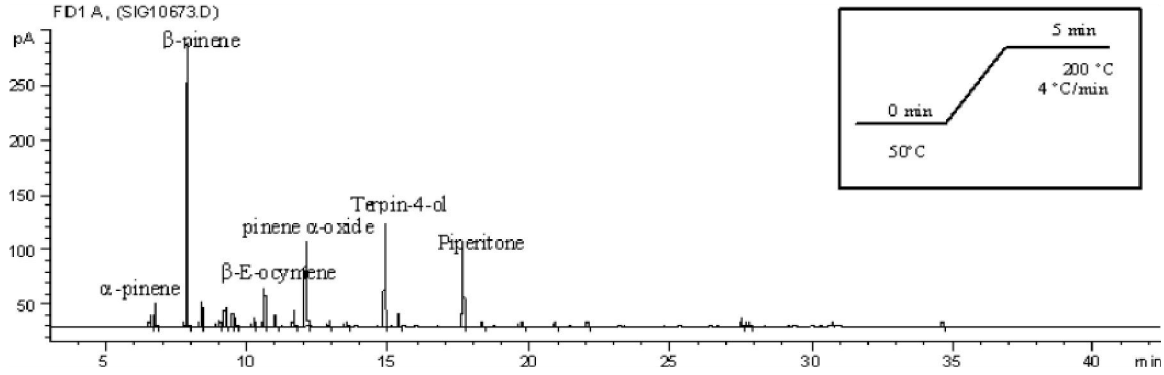

Figure 2 : Chromatographic profiles of essential oils in the branches of *juniperus oxycedrus*

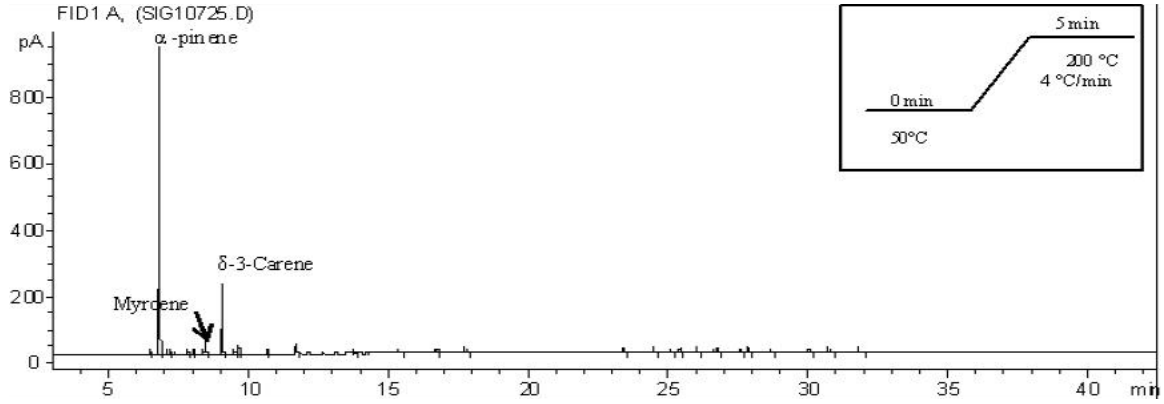

Figure 3 : Chromatographic profiles of essential oils in the branches of *juniperus phoenicea*

TABLE 2 : Antioxidant activity of essential oils in the branches of *juniperus thurifera, J. oxycedrus* and *J. phoenicea* in Morocco

Plants	*J. thurifera*	*J. oxycedrus*	*J. phoenicea*	BHT
DPPH, IC50 (μg/ml)	4.75	4.90	5.50	1.13

IC50: Concentration corresponding to 50% of inhibition (antioxidant activity)

and 19.2% respectively) and for α-terpenyle acetate (15.5 and 12.5% respectively).

The results obtained are in agreement with those announced by Adams[25] from the analysis of *Juniperus* genus, in which pinenes are generally dominant. These compounds can constitute important molecules discriminating various species of juniper[25]. Moreover, they can also be useful as biochemical markers in the genetic study of forests, for the determination of geographical variability of conifer species and for the identification of families and clones[26, 27, 28, 19, 30].

Antioxidant activity of essential oils

The measurement of absorbance (optical density C) was taken by spectrophotometry with 517 nm. From the values obtained, we calculated the percentages of inhibition by using the formula given previously and plotted the graphs in Figure 4, representing the variations in inhibition percentage according to the concentration of essential oils. We determined the concentration graphically corresponding to 50% of inhibition (IC_{50}), and to the antioxidant activity of studied essential oils. By comparing the results of the antioxidant activity with that of compounds of reference (BHT), we obtain the values reported in TABLE 2.

It is noted that the essential oils of thuriferous juniper appear antioxidant at a higher level than that of red junipers and Phoenicean junipers. This can be explained by their chemical profiles whose content in oxygenated terpenes is approximately 42%, compared with only 14% for the essential oils of red juniper and approximately 7% for the Phoenicean juniper. This result is also in agreement with several works[31,32,33] which showed that the antioxidant

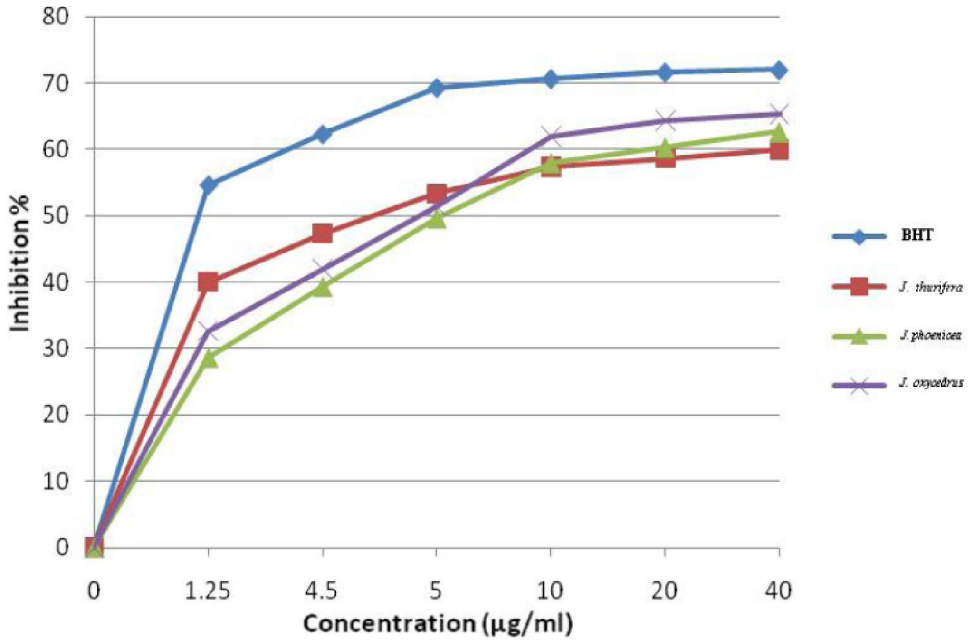

Figure 4 : Antioxydant activity of the essential oils of the branches of *juniperus thurifera, juniperus oxycedrus* and *juniperus phoenicea* and the BHT

properties are in close relationship to the presence of compounds comprising hydroxyl groups. However, it is difficult to assign this activity to the only oxygenated compounds because of the chemical complexity of essential oils which can generate a synergy effect between the various compounds.

By studying the antioxidant effect of the essential oils from the fruits of *J. oxycedrus* in Lebanon, Loizzo and al. (2007)[15] found a value of IC_{50} of 7.42 µg/ml, which remains lower than antioxidant capacity of essential oils from our three species of junipers. A study carried out by Bouzouita and al. (2008)[34] on the antioxidant activity of the essential oils of *J. phoenicea* in Tunisia showed that it has also a good antioxidant potential by reference to the usual antioxidant δ-tocopherol. Emami and al. (2007)[35] also identified an important antioxidant effect in several species of the *Juniperus* genus originating from Iran.

The results obtained show that essential oils of junipers reduced free radical DPPH with a good antioxidant potential by reference to the BHT whose value of IC_{50} is 1.13µg/ml. This value of IC50 expresses the concentration of essential oils required to reduce radical DPPH in solution of 50%.

CONCLUSION

The essential oils of branches from the three studied species of junipers are very rich in pinenes: β-pinene (36.26%) from *J. thurifera*, α-pinene from *J. oxycedrus* (52.13%) and *J. phoenicea* (64.19%). In comparison with an antioxidant of reference (BHT), essential oils from the three species of junipers showed that they are equipped with a good antioxidant activity. This characteristic deserves further investigations for the use of these oils for therapeutics ones, cosmetics and agribusiness industries.

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